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November 15, 2001

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Billing No. 23893-7180

RECEIVED
APR 17 2002
OFFICE OF PETITIONS

Randy Jay McCoy
3212 Breton Avenue
Davis, CA 95616

Re: U.S. Patent Application Serial No. 09/966,982
For: NOVEL STRAIN OF STREPTOMYCES FOR CONTROLLING PLANT DISEASES
Applicants: Lori Jo Lehman et al.
Our Ref.: AQ 2018.20

Dear Dr. McCoy:

Enclosed are the following documents in connection with the above patent application:

1. Declaration of Utility Patent Application for execution by you where indicated;

2. Joint Assignment for execution by you where indicated. Please be sure that the Assignment is executed after the Declaration has been executed; and

3. A copy of the application as filed.

Please review the application as filed. After reviewing the application, please sign the Declaration for Utility Patent, preferably in **blue ink**. Minor typographical errors and the like can be corrected by amendment.

Under U.S. law, the applicant and/or inventors have a continuing duty to disclose to the U.S. Patent Office information of which they are aware that may be material to the examination of the application. Accordingly, if you become aware of additional references or other information (e.g., possible prior public uses or sales of the invention i the United States, prior invention by another, knowledge derived from others in making the invention, and inventorship conflicts) during the pendency of this application, please notify us.

Please have the documents executed and returned to us at your earliest convenience so that we can file them before **December 23, 2001**. For your convenience we have enclosed a stampted envelope addressed to McCutchen.

Please do not hesitate to contact me if you have any questions.

Very truly yours,

Carol M. Gruppi

cc: Antoinette F. Konski (w/o enc.)

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AQ 2018.20

DECLARATION FOR UTILITY PATENT APPLICATION

AS BELOW-NAMED INVENTORS, WE HEREBY DECLARE THAT:

Our residence, post office address, and citizenship are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: **Novel Strain Of Streptomyces For Controlling Plant Diseases** the specification of which is attached hereto unless the following box is checked:

☑ was filed on **September 27, 2001** as United States Application Serial No. 09/966,982.

WE HEREBY STATE THAT WE HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

We acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
			☐Yes ☐No

We hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date

We hereby claim the benefit under 35 U.S.C. § 120 of the United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Application Serial No.	Filing Date	Status
09/671,943	September 27, 2000	Pending

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title of 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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ASSIGNMENT (JOINT)

THIS ASSIGNMENT, by **Lori Jo Lehman, Randy Jay McCoy, Cai-yao Yuan, Denise Carol Manker, Jimmy Ensio Orjala, Pamela Gail Marrone** and **Jorge Isaac Jimenez Santamaria** (hereinafter referred to as the assignors), residing at **1218 Las Encinas Court, Vacaville, CA 95687, 3212 Breton Avenue, Davis, CA 95616, 1213 Chestnut Lane, Davis, CA 95616, 3037 Prado Lane, Davis, CA 95616, 2905 Lillard Drive, Davis, CA 95616, 3333 Victoria Place, Davis, CA 95616 and 2326 V Street, Apt. #4, Sacramento, CA 95818,** respectively, witnesseth:

WHEREAS, said assignors have invented certain new and useful improvements in **Novel Strain Of Streptomyces For Controlling Plant Diseases**, set forth in a patent application for the United States, bearing Application No. **09/966,982**, filed on **September 27, 2001**; and

WHEREAS, **AgraQuest, Inc.**, a corporation duly organized under and pursuant to the laws of **Delaware** and having its principal place of business at **1530 Drew Avenue, Davis, California 95616** (hereinafter referred to as the assignee) is desirous of acquiring the entire right, title and interest in and to said inventions and said application and any applications for Letters Patent which claim priority of the above-mentioned application under 35 U.S.C. § 119(e), alone or in combination with 35 § U.S.C. 120 (hereinafter collectively referred to as an application for "Letters Patent") of the United States;

NOW, THEREFORE, in consideration of One Dollar ($1.00) and other good and sufficient consideration, the receipt of which is hereby acknowledged, said assignors have sold, assigned, transferred and set over, and by these presents does sell, assign, transfer and set over, unto said assignee, its successors, legal representatives and assigns, the entire right, title and interest in and to the above-mentioned inventions, application for Letters Patent, and the same to be held and enjoyed by said assignee, for its own use and the use of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted, as fully and entirely as the same would have been held and enjoyed by the assignors, had this sale and assignment not been made.

AND for the same consideration, said assignors hereby covenant and agree to and with said assignee its successors, legal representatives and assigns, that, at the time of execution and delivery of these presents, said assignors are the joint and lawful owners of the entire right, title and interest in and to said inventions and the application for Letters Patent above-mentioned, and that the same are unencumbered and that said assignors have good and full right and lawful authority to sell and convey the same in the manner herein set forth.

AND for the same consideration, said assignors hereby covenant and agree to and with said assignee, its successors, legal representatives and assigns, that said assignors will, whenever counsel of said assignee, or the counsel of its successors, legal representatives and assigns, shall advise that any proceeding in connection with said applications for Letters Patent and any patents to be obtained thereon, granted thereon is lawful and desirable, sign all papers and documents,

take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement and defense of Letters Patent for said inventions, without charge to said assignee, its successors, legal representatives and assigns, but at the cost and expense of said assignee, its successors, legal representatives and assigns.

AND said assignors hereby request the Commissioner for Patents to issue said applications for Letters Patent and patents granted thereon of the United States to said assignee as the assignee of said inventions and the Letters Patent to be issued thereon for the sole use of said assignee, its successors, legal representatives and assigns.

Date	Name: **Lori Jo Lehman**
Date	Name: **Randy Jay McCoy**
Date	Name: **Cai-yao Yuan**
Date	Name: **Denise Carol Manker**
Date	Name: **Jimmy Ensio Orjala**
Date	Name: **Pamela Gail Marrone**
Date	Name: **Jorge Isaac Jimenez Santamaria**

Attorney Docket No. AQ 2018.20

EL832926645US

NOVEL STRAIN OF STREPTOMYCES FOR CONTROLLING PLANT DISEASES

INVENTORS:

Lori Jo Lehman et al.
Randy Jay McCoy
Cai-yao Yuan
Denise Carol Manker
Jimmy Ensio Orjala
Pamela Gail Marrone
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A NOVEL STRAIN OF *Streptomyces* FOR CONTROLLING PLANT DISEASES

Cross-Reference to Related Applications

This application is a continuation-in-part of U.S. Application Serial No. 09/671,943 filed September 27, 2000. The contents of this application is hereby incorporated by reference into the present disclosure.

Field of the Invention

The present invention is in the field of biopesticides.

Background of the Invention

For a number of years, it has been known that various microorganisms exhibit biological activity so as to be useful to control plant diseases. Although progress has been made in the field of identifying and developing biological pesticides for controlling various plant diseases of agronomic and horticultural importance, most of the pesticides in use are still synthetic compounds. Many of these chemical fungicides are classified as carcinogens by the EPA and are toxic to wildlife and other non-target species. In addition, pathogens may develop resistance to chemical pesticides (Schwinn *et al.*, 1991).

Biological control offers an attractive alternative to synthetic chemical fungicides. Biopesticides (living organisms and the compounds naturally produced by these organisms) can be safer, more biodegradable, and less expensive to develop.

The actinomycetes, including the streptomycetes, are known producers of antifungal metabolites (Lechavalier and Waksman, 1962; Lechavalier, 1988). Several actinomycete-produced antibiotics are routinely used in an agricultural setting such as streptomycin and terramycin for fire blight control.

Streptomycetes have demonstrated both *in vitro* and *in vivo* activity against plant pathogens. Axelrood *et al.* (1996) isolated 298 actinomycetes from Douglas-fir roots. Approximately 30% of these strains demonstrated antifungal activity against Fusarium, Cylindrocarpon, and/or Pythium *in vitro*. Yuan and Crawford (1995) reported that *Streptomyces lydicus* WYEC108 showed both strong *in vitro* antifungal

activity and inhibition of Pythium root rot in pot tests with pea or cotton seed. Reddi and Rao (1971) controlled Pythium damping-off in tomatoes and Fusarium wilt of cotton with *Streptomyces ambofaciens*. Rhizoctonia root rot was controlled by *Streptomyces hygroscopicus* var. *geldanus* (Rothrock and Gottlieb, 1984). These authors reported that the control was dependent on the in situ geldanamycin concentration produced by this strain. The same authors also saw protection of soybeans from *Phytophthora megasperma* var. *sojae* by *Streptomyces herbaricolor* and *Streptomyces coeruleofuscus* (1984). Chamberlain and Crawford (1999) saw *in vitro* and *in vivo* antagonism of turfgrass fungal pathogens by *S. hygroscopicus* strain YCED9. Crawford (1996) patented the use of this strain to control plant pathogens in US patent 5, 527,526. Suh (1998) patented 2 *Streptomyces* sp. that were active against *Rhizoctonia solani* and *Phytophthora capsici*. A *Streptomyces griseoviridis* product against *Fusarium* spp. and other soil pathogens is on the market as Mycostop™.

Summary of the Invention

A novel antibiotic-producing *Streptomyces* sp. is provided that exhibits antifungal activity only on certain specific plant pathogens. Also provided is a method of treating or protecting plants from fungal infections comprising applying an effective amount of an antibiotic-producing *Streptomyces* sp. having all the identifying characteristics of NRRL Accession number B-30145. The invention also relates to fungicidal compositions comprising this novel *Streptomyces* strain and the antibiotics and metabolites produced by this strain either alone, or in combination with other chemical and biological pesticides.

The antibiotic-producing *Streptomyces* sp. can be provided as a suspension in a whole broth culture or as an antibiotic-containing supernatant obtained from a whole broth culture of an antibiotic-producing *Streptomyces* sp. Also provided is a novel butanol-soluble antibiotic that exhibits specific antifungal activity and a process for isolating the novel butanol-soluble antibiotic.

Brief Description of the Figures

Figure 1A is the analytical HPLC chromatogram of active fraction 6. (Microsorb C18, 10cm x 4.6mm, 100Å, flow rate 1mL/min, UV detection at 220nm, acetonitrile + 0.05% TFA/water + 0.05% gradient as follows: 0-30 min, 5-65%; 30-40 min, 65-100%; 40-45 min, 100%).

Figure 1B is the UV spectrum of the active peak eluting at 14.755 minutes in the chromatogram described in 1A.

Figure 2A is the analytical HPLC chromatogram of active fraction 7 under the same conditions described in 1A.

Figure 2B is the UV spectrum of the active peak eluting at 16.146 minutes in the chromatogram described in 2A.

Figure 3 is the C-8 HPLC chromatogram of the methanol eluate from the Diaion HP-20 resin step described in Method B. (HP Zorbax Eclips XDB-C8 column, 5μm, 150 x 4.6mm, flow rate 0.8 mL min, UV detection at 220 nm, chart speed 2mm/min. Solvent A, 25:5:70 acetonitrile/methanol/water. Solvent B, 65:5:30 acetonitrile/methanol/water. Gradient: 100% A at 0 minutes increased to 3% B over 20 minutes.)

Figure 4 is the ^{1}H NMR spectrum (400MHz, CD_3OD) of the semi-pure active metabolite obtained from purification method A.

Figure 5 is the ^{13}C NMR spectrum (100MHz, CD_3OD) of the semi-pure active metabolite obtained from purification method A.

Figure 6 is the LC ESI-MS (Liquid Chromatography ElectroSpray Impact – Mass Spectrum) of Peak A obtained from purification method B. (Microsorb C18, 10cm x 4.6mm, 100Å, flow rate 1mL/min, acetonitrile + 0.02% TFA/water + 0.02% gradient as follows: 0-30 min, 5-65%; 30-40 min, 65-100%; 40-45 min, 100%).

Figure 7 is the LC ESI-MS (Liquid Chromatography ElectroSpray Impact – Mass Spectrum) of Peak B obtained from purification method B. (Microsorb C18, 10cm x 4.6mm, 100Å, flow rate 1mL/min, acetonitrile + 0.02% TFA/water + 0.02% gradient as follows: 0-30 min, 5-65%; 30-40 min, 65-100%; 40-45 min, 100%).

Detailed Description

The present invention provides a novel strain of *Streptomyces sp.* or mutants thereof with antifungal activity only on specific plant pathogens such as *Alternaria*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Sclerotinia*. This novel strain was deposited with the NRRL on July 20, 1999 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure under Accession No. B-30145. The invention also includes methods of preventing and treating fungal diseases in plants using such bacterial strains or antibiotic-containing supernatants or pure antibiotics obtained from such bacterial strains. The invention also includes a butanol soluble antifungal antibiotic with a molecular weight of less than 10,000 daltons, with stability to base and to heat treatment of 1 hour at 80°C and lability to acid treatment.

Definitions

The singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and agriculturally acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for applying the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

As used herein, "biological control" is defined as control of a pathogen or insect by the use of a second organism. Known mechanisms of biological control include enteric bacteria that control root rot by out-competing fungi for space on the surface of the root. Bacterial toxins, such as antibiotics, have been used to control

pathogens. The toxin can be isolated and applied directly to the plant or the bacterial species may be administered so it produces the toxin *in situ*.

The term "fungus" or "fungi" includes a wide variety of nucleated spore-bearing organisms that are devoid of chlorophyll. Examples of fungi include yeasts, molds, mildews, rusts, and mushrooms.

The term "bacteria" includes any prokaryotic organism that does not have a distinct nucleus.

"Pesticidal" means the ability of a substance to increase mortality or inhibit the growth rate of plant pests.

"Fungicidal" means the ability of a substance to increase mortality or inhibit the growth rate of fungi.

"Antibiotic" includes any substance that is able to kill or inhibit a microorganism. Antibiotics may be produced by a microorganism or by a synthetic process or semisynthetic process. The term, therefore, includes a substance that inhibits or kills fungi for example, cycloheximide or nystatin.

"Antifungal" includes any substance that is able to kill or inhibit the growth of fungi.

The term "culturing" refers to the propagation of organisms on or in media of various kinds. "Whole broth culture" refers to a liquid culture containing both cells and media. "Supernatant" refers to the liquid broth remaining when cells grown in broth are removed by centrifugation, filtration, sedimentation, or other means well known in the art.

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations. In terms of treatment and protection, an "effective amount" is that amount sufficient to ameliorate, stabilize, reverse, slow or delay progression of the fungal or bacterial disease states.

"Positive control" means a compound known to have pesticidal activity. "Positive controls" include, but are not limited to commercially available chemical pesticides. The term "negative control" means a compound known not to have pesticidal activity. Examples of negative controls are water or ethyl acetate.

The term "solvent" includes any liquid that holds another substance in solution. "Solvent extractable" refers to any compound that dissolves in a solvent and which then may be isolated from the solvent. Examples of solvents include, but are not limited to, organic solvents like ethyl acetate.

The term "metabolite" refers to any compound, substance or byproduct of a fermentation of a microorganism that has pesticidal activity. Antibiotic as defined above is a metabolite specifically active against a microorganism.

The term "mutant" refers to a variant of the parental strain as well as methods for obtaining a mutant or variant in which the pesticidal activity is greater than that expressed by the parental strain. The "parent strain" is defined herein as the original *Streptomyces* strain before mutagenesis. To obtain such mutants the parental strain may be treated with a chemical such as N-methyl-N'-nitro-N-nitrosoguanidine, ethylmethanesulfone, or by irradiation using gamma, x-ray, or UV-irradiation, or by other means well known to those practiced in the art.

A "composition" is intended to mean a combination of active agent and another compound, carrier or composition, inert (for example, a detectable agent or label or liquid carrier) or active, such as an adjuvant. Examples of agricultural carriers are provided below.

We describe a novel antibiotic-producing strain of *Streptomyces* sp. NRRL No. B-30145 and mutants thereof that have antifungal activity only on specific plant pathogens. Also provided is a supernatant isolated from the culture as well as a composition comprising the culture. In a further aspect, the compositions further comprise at least one chemical or biological pesticide.

A metabolite produced by the *Streptomyces* sp. strain is also provided by this invention. The metabolite exhibits activity against plant pathogenic fungi and is heat and base stable, is acid labile and has a molecular weight of less than 10,000 daltons. By way of example, the metabolite may have a molecular weight [$M + H^+$] between about 925 to between about 865.

The one or more metabolites produced by the *Streptomyces* sp. strain exhibit UV absorption between about 215 nm and 220 nm. The metabolite may be comprised of a variety of molecules including, but not limited to, propargyl alcohol segments

[C=C-CH(OH)], oxygenated methine carbons (X-CH-Y) or a sugar moiety. By way of example, the metabolite may comprise at least two propargyl segments, several oxygenated methine carbons (by way of example, e.g., 5 to 10) and /or a sugar moiety. Alternatively, the one or more metabolites produced by the *Streptomyces* sp. strain may share the same carbon skeleton and differ in degree of oxygenation.

The present invention also provides antifungal compositions comprising a metabolite produced by *Streptomyces* and isolated according to a method comprising:

(a) loading a whole broth culture of *Streptomyces* sp. strain NRRL No. B-30145 or mutants thereof having all the identifying characteristics of NRRL No. B-30145 onto a non-ionic absorbent polymeric resin;

(b) eluting the metabolite with an alcohol;

(c) screening the eluate of step (b) with a bioassay for fractions of the eluate exhibiting antifungal activity;

(d) loading the fractions of the eluate exhibiting antifungal activity of step (c) on a HPLC column; and

(e) eluting the metabolite with an organic solvent.

The method may further comprise washing the resin with water prior to step (b) and screening the eluate of step (e) with a bioassay to select the fractions exhibiting antifungal activity.

The whole broth culture of step (a) may be freeze dried and re-suspended with an aqueous solution (e.g., water) prior to adding to the non-ionic absorbent polymeric resin. In a preferred embodiment the whole broth culture added to the resin is a homogenized cell-free whole broth culture. Examples of non-ionic absorbent polymeric resin that may be used include, but are not limited to, Supelco Sepabead SP-207 or Supelco Diaon HP-20.

The eluent used to remove the metabolite in step (b) may be an alcohol or a gradient of aqueous alcohol. By way of example, methanol or a gradient of aqueous methanol may be used as the eluent (e.g., Example 6).

The bioassay of step (c) may be any assay which evaluates antifungal activity. Examples of such bioassays include but are not limited to, the agar diffusion assay or

slide germination assay. For example, the bioassay may be a germination assay with *Monilinia fructicola* and/or *Alternaria brassicicola.*

Examples of an HPLC column that may be used in step (d) include, but are not limited to, C-18 or C-8. Examples of the organic solvent that may be used to remove the metabolite from the HPLC column include, but are not limited to, an acetonitrile – water gradient (e.g., Example 6).

The metabolite can also be formulated as a composition, with a carrier or alternatively, with at least one chemical or biological pesticide.

In order to achieve good dispersion and adhesion of compositions within the present invention, it may be advantageous to formulate the whole broth culture, supernatant and/or metabolite/antibiotic with components that aid dispersion and adhesion. Suitable formulations will be known to those skilled in the art (wettable powders, granules and the like, or can be microencapsulated in a suitable medium and the like, liquids such as aqueous flowables and aqueous suspensions, and emulsifiable concentrates). Other suitable formulations will be known to those skilled in the art.

The strain, culture, supernatant and isolated metabolite are useful to protect or treat plants, fruit, and roots from fungal infections by applying an effective amount of the active formulation to the plant, fruit or root. The formulations are particularly suited to treat or prevent infections caused by a fungus selected from the group consisting of *Alternaria solani*, *Botrytis cinerea, Rhizoctonia* sp., *Sclerotinia* sp., and *Phytophthora* sp.

All patents and publications cited herein are incorporated by reference. Full bibliographic citations for these may be found at the end of the specification, immediately preceding the claims.

The following examples are provided to illustrate the invention. These examples are not to be construed as limiting.

EXAMPLES

Example 1

Characterization of Strain NRRL No. B-30145

NRRL No. B-30145 was identified based on 16S rRNA sequencing. The protocol used to generate the 16S rRNA gene data sequence (Acculab Customer Handbook v. 1.0) is described as follows.

The 16S rRNA gene is PCR amplified from genomic DNA isolated from bacterial colonies. Primers used for the amplification correspond to *E. coli* positions 005 and 531. Amplification products are purified from excess primers and dNTPs using Microcon 100 (Amicon) molecular weight cut-off membranes and checked for quality and quantity by running a portion of the products on an agarose gel.

Cycle sequencing of the 16S rRNA amplification products is carried out using AmpliTaq FS DNA polymerase and dRhodamine dye terminators. Excess dye-labeled terminators were removed from the sequencing reactions using a Sephadex G-50 spin column. The products are collected by centrifugation, dried under vacuum and frozen at –20°C until ready to load. Samples are re-suspended in a solution of formamide/blue dextran/EDTA and denatured prior to loading. The samples are electrophoresed on an ABI Prism 377 DNA Sequencer. Data are analyzed using PE/Applied Biosystem's DNA editing and assembly software. Once obtained, sequences are compared against PE/Applied Biosystem's MicroSeq™ database using MicroSeq™ sequence analysis software. Sequences are also compared to the GenBank and Ribosomal Database Project (RDP).

The result of the 16S rRNA sequencing identified NRRL No. B-30145 as a *Streptomyces* sp. This strain may belong to the species *S. mashuensis* (formerly *Streptoverticillium mashuense*) or a related species, as suggested by the sequencing results. The best match was *Streptomyces mashuensis* with a 98% match score.

Example 2

Activity of NRRL No. B-30145 against plant pathogens in *in-vitro* culture (zone assay).

NRRL No. B-30145 was tested against an array of different plant pathogens utilizing two different *in-vitro* assays. The agar diffusion (zone) assay consists of applying either plant pathogen spores over the surface of an agar medium to create an entire lawn of growth or utilizing a mycelial agar plug placed in the center of the petri dish that will grow and colonize the agar. Circular wells approximately 7.0mm in diameter are removed from the agar using a pipette attached to a vacuum pump. Fermentation samples of NRRL No. B-30145 are added to each well along with known standards and water checks. Plates are incubated for three to four days under environmental conditions conducive for each pathogen. Results consist of a zone of no pathogen growth around the well or a greatly reduced amount of pathogen growth around the well or no affect. The size and type of zone is recorded for each sample. Results for NRRL No. B-30145 in agar diffusion assays are presented in Table 1. Results within agar diffusion were variable; diffusion through agar may be inhibited.

Table 1: Activity of NRRL No. B-30145 against selected plant pathogens in the agar diffusion (zone) assay.

Alternaria brassicicola	No Zone / Weak Activity
Botrytis cinerea	Weak Activity
Monilinia fructicola	No Zone
Phytophthora capsici	Moderate activity
Pythium sp.	Weak Activity
Colletotrichum acutatum	No Zone
Rhizoctonia solani	No Zone
Sclerotinia sclerotiorum	No Zone

The second type of *in-vitro* assay performed to test the pathogen spectrum of NRRL No. B-30145 was the slide germination assay. Fermentation samples of NRRL No. B-30145 at various dilutions were added to glass depression slides (25mm x

75mm with 18mm diameter depression 1.75mm deep) and an equal volume of pathogen spores were mixed with the sample. Slides were incubated on moistened paper towels in sealed plastic boxes at room temperature overnight. Results are determined by observing the fermentation sample / spore suspension sample using a compound microscope at 100X. Typical results consist of lack of germination of the pathogen propagule or greatly reduced germination and/or growth. In addition, various types of malformations of the initial growth from the pathogen spores may occur. The spectrum of activity of NRRL No. B-30145 is presented in Table 2. Complete inhibition of spore germination occurred at low concentrations of fermentation samples.

Table 2: Activity of NRRL No. B-30145 against selected plant pathogens in the slide germination assay.

Alternaria brassicicola	No Germination
Alternaria dauci	No Germination
Botrytis cinerea	No Germination
Monilinia fructicola	No Germination

Example 3

Activity of NRRL against plant pathogens in plant bioassay tests.

Activity of NRRL No. B-30145 was tested against tomato late blight (*Phytophthora infestans*), tomato early blight (*Alternaria solani*), gray mold (*Botrytis cinerea*), turf brown patch (*Rhizoctonia* sp.), and peanut southern blight (*Sclerotinia minor*). All tests were conducted under controlled environment in the laboratory with plant material grown from seed under typical commercial greenhouse conditions.

Tomato Late Blight – *Phytophthora infestans*

The pathogen is grown on rye agar in standard petri dishes at 16°C in the dark. Sporangia are collected by flooding the plate with water and scraping the mycelium to dislodge the sporangia. The sporangial suspension is passed through cheesecloth, quantified and adjusted to 1.0×10^{-4}. Tomato seedlings at the 3rd to 5th leaf stage are

sprayed to run-off with the fermentation sample of NRRL No. B-30145 using an artist airbrush at 40psi. Treated seedlings are allowed to air dry at room temperature for at least two hours then inoculated with the sporangial suspension by lightly spraying the upper surfaces of the tomato seedlings using a hand held sprayer. Inoculated seedlings are placed in solid bottom flats filled with water and then are covered with a plastic dome to maintain leaf wetness. Flats are incubated at 20°C with a 14-hr photoperiod for four days continuously covered by the plastic domes. Seedlings are then rated based on a disease rating scale from 0 – 5 with 0 equaling no symptoms, and 5 equaling 75% or more of the foliage colonized by the pathogen. A typical example of a late blight test is presented in Table 3.

Table 3: Results of NRRL No. B-30145 treated tomato seedlings against the late blight pathogen *Phytophthora infestans*.

Treatment	Ave.D.I.	Replications 1 - 4			
Sample 990702	1.1	1.0	0.5	2.0	1.0
Sample 990709	1.1	1.0	2.0	1.0	0.5
Sample 990825	1.3	1.5	1.0	1.5	1.0
Sample 990913	1.0	1.0	1.0	1.5	0.5
Quadris 30ppm	0.1	0	0.5	0	0
Water Check	4.3	4.0	4.0	5.0	4.0

Samples are different fermentations of NRRL No. B-30145.
D.I. is Disease Index.

Tomato Early Blight – *Alternaria solani*

The pathogen is first grown on commercial Difco potato dextrose agar (PDA) at 22-25°C under 14-hour lights until the entire plate is covered. The fungus and the agar medium is then cut into small squares approximately 10mm square and placed fungus side up on a specialized sporulation medium (S-Medium: 20g sucrose, 20g calcium carbonate, 20g Bacto-agar per liter). The S-Media plates are flooded with a thin layer of water and incubated 2-3 days at 22 – 25°C under 14-hour lights until full

sporulation of the pathogen occurs. Plates are then flooded with water and the agar squares are scraped from the plate. The suspension is passed through cheesecloth and the spores are quantified and adjusted to $1.0X10^5$. Tomato seedlings at the 3rd to 5th leaf stage are then sprayed until run-off using an artist airbrush as described previously. Treated seedlings are allowed to dry and then inoculated with the spore suspension. Seedlings are placed in flats and covered as described previously and incubated at 25°C with a 14-hour photoperiod. Seedlings are rated based on a scale of 0 – 5 as previously described. Results from a typical test are presented in Table 4.

Table 4: Activity of NRRL No. B-30145 against the early blight pathogen *Alternaria solani.*

Treatments	Ave D.I.	Replications			
Test-1					
Sample 990216	1.0	2.0	0.5	0.5	
Quadris 60 ppm	1.8	1.5	2.5	1.5	
Water Check	4.0	3.0	4.0	5.0	
Test-2					
Sample 990216	1.1	1.5	1.0	1.0	1.0
Water Check	4.5	5.0	4.0	4.0	5.0

D.I. is Disease Index.

Pepper Gray Mold – *Botrytis cinerea*

The pathogen is grown on standard PDA under a 14-hour photoperiod at 22°C until the fungal growth has completely covered the plate (7-9 days). Spores are collected by flooding the plate with water and then gently scraping with a spatula to dislodge the spores. The spore suspension is passed through cheesecloth and quantified and adjusted to $1.5X10^6$. Pepper seedlings are grown until the 4th to 6th true leaf stage and fermentation samples are sprayed on the upper leaf surfaces using an artist airbrush as described previously. Treated seedlings are inoculated, placed in flats and covered with plastic domes. Flats are placed at 20°C under continuous

darkness for 2.5 days. Seedlings are rated on a 0 – 5 scale as described previously. Table 5 depicts results from two typical tests.

Table 5: Activity of NRRL No. B-30145 against *Botrytis cinerea*

Treatment	Ave D.I.	Replications			
Test -1					
Sample 990216	1.4	1.5	1.5	1.5	1.0
Break 20ppm	0.1	0	0	0.5	0
Water Check	4.0	4.0	4.0	3.0	5.0
Test-2					
Sample 990216	1.9	1.5	2.0	2.0	2.0
Break 20ppm	0.8	0	1.5	1.0	0.5
Water Check	4.5	4.0	5.0	5.0	4.0

D.I. is Disease Index.

Turf brown patch-*Rhizoctonia* sp.

Two ml of fermentation sample was added to each cell of a 6-cell pot of one-month old turf seedlings (Bentgrass). A 4 mm mycelial plug of a 2-3 day old culture of *Rhizoctonia* sp. was placed under the soil surface. Each treatment was replicated 6 times. Inoculted pots were placed in plastic flats and covered with a plastic dome. The flats were placed on a light rack (16Hr/day) and incubated at room temperature. Disease severity was evaluated after 5-6 days incubation and compared with the water treated control. The results indicated that NRRL No. B-30145 has a suppressive activity against Rhizoctonia (Table 6).

Table 6. The efficacy of NRRL No. B-30145 on turf disease caused by *Rhizoctinia* sp.

Treatment	Dilution Factor	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
30145	1x	+*	+	+	+	+	+
30145	½ x	+	+	+	+	+	+
Water		+++	+++	+++	+++	+++	+++

* "+" = light symptoms, "+++"=severe symptoms

Peanut southern blight-*Sclerotinia minor*

Peanut seedlings at the first 2-leaf stage were treated with NRRL No. B-30145 and a 4-mm mycelial plug is placed on the base of each stem after the treated plants dried. Inoculated plants were incubated in a dew chamber for 2 days before being placed in a plastic flat sealed with a cover dome. The flat was incubated on a light rack (16Hr/day) at room temperature for 10 days. Disease severity was assessed by comparing the treated with the water control. The results (Table 7) indicated NRRL No. B-30145 whole broth at 1x has some controlling activity against *Sclerotinia minor.*

Table 7. The efficacy of NRRL No. B-30145 on peanut Sclerotinia blight.

Treatment	Dilution Factor	Rep 1	Rep 2	Rep 3
30145	1x	+/-	0	+/-
30145	½ x	++	++	+
30145	¼ x	0	++	++
Water		+++	+++	+++

*"+/- " indicates strong suppression, 0 indicates no infection, "+" = light symptoms, "+++"=severe symptoms,.

Example 5

Antifungal metabolite produced by NRRL No. B-30145.

The whole broth of NRRL No. B30145 was partitioned into ethyl acetate, butanol and aqueous fractions. Each fraction was tested against *Alternaria brassicicola* in a spore germination assay. The *Alternaria* spores were germinated in the presence of each sample in depression microscope slides containing 40 µl of sample and 20 µl of pathogen spores. Approximately 16 hours later the spores are

observed under a microscope to see if they have germinated. No germination (score of 0) compared to the water control (100% germination and growth=score of 5) indicates activity of the sample being tested. Results of the *Alternaria* germination assay with different NRRL No. B-30145 fractions are shown below (score on a 0 to 5 rating as above).

Fraction	**Score**		
	Rep 1	Rep 2	Rep3
Ethyl acetate	3	nd	4
n-butanol	0	0.2	1
Aqueous	0	5	5
Whole broth	0	0.2	0
Water Check	5	5	5

The metabolite is clearly in the butanol soluble fraction and is not readily extractable in ethyl acetate. Other characteristics of the metabolite were determined. The molecule was shown to pass through a 10,000 molecular weight cut off filter indicating the metabolite is smaller than 10,000 daltons. The activity was not lost when treated with base or upon heating to 80 degrees C for one hour. The activity was lost when treated with acid (the score against *Alternaria* increased from 0 to 5).

Fractionation of the butanol extract on octadecylsilane bonded to silica gel (ODS) flash chromotography using an acetonitrile (ACN)/water gradient with 0.01 % trifluoroacetic acid (TFA) yielded an active fraction eluting with 50% acetonitrile/water with 0.01 % TFA. Fractions were tested in an *Alternaria* germination assay for activity (0-5 rating scale).

Fraction	Score
ODS 10% ACN	4
ODS 20% ACN	5
ODS 50% ACN	0.5
ODS 100% ACN	5
Water Check	5

Further purification by ODS HPLC yielded 2 active components (Fraction 6 and 7) from an isocratic elution with 31% acetonitrile in water with 0.02 % TFA).

Fraction	Score
HPLC Fr.1	5
HPLC Fr.2	5
HPLC Fr.3-5	4
HPLC Fr.6	3
HPLC Fr.7	2
HPLC Fr.8	5
HPLC Fr.9	5
Water Check	5

The HPLC chromatogram of the active 50% acetonitrile/water with 0.01 % TFA flash chromotography fraction and the HPLC chromatograms of the active fractions 6 and 7, including UV spectra of the active principles, are shown in Figures 1 and 2.

NRRL No. B-30145 most closely matched *Streptomyces mashuensis* by 16S RNA sequencing. Unlike the antibacterial metabolites typically associated with *S. mashuensis*, the fungicidal activity of NRRL No. B-30145 was extractable with butanol. *S. mashuensis* is known to produce streptomycin, which is a water-soluble antibacterial compound. Another antibiotic produced by *S. mashuensis*, monazomycin (Akasaki *et al.* 1963), does not display a shoulder at 215 –220 nm as does the fungicidal active fractions of NRRL No. B-30145.

Antifungal compounds have also been found in the closely related and possibly synonymous species *Streptomyces griseocarneum* (American Type Culture Collection). These include porfiromycin (Claridge *et al.*, 1986), a purple compound whose corresponding UV spectrum is not seen in the active fraction of NRRL No. B-30145 and the *Heptaenes trichomycin* (Komori and Morimoto, 1989) and *griseocarnin* (Campos *et al.*, 1974), whose corresponding UV spectra are also not present in the active fraction. The fungicidal active is also not neutramycin, which is extractable with ethyl acetate (Mitscher and Kunstmann, 1969).

Example 6

Additional Methods for further purification of the Antifungal metabolite of NRRL No. B-30145

Method A

The freeze-dried whole broth culture was re-suspended in water (2. 0 L) and loaded onto a column containing a non-ionic polymeric resin (Supelco Sepabead SP-207; 26 x 3.0 cm) equilibrated in water. The column was washed with water (200 mL) and then with a gradient of aqueous methanol as follows: (1) 20:80 methanol/water (200 mL), (2) 40:60 methanol/water (200 mL), (3) 60:40 methanol/water (200 mL), 80/20 methanol/water (200 mL), and (5) methanol (200 mL).

Bioassay results (germination assay with *Monilinia fructicola* and/or *Alternaria brassicicola*) indicated that all fractions were active. Each fraction was individually fractionated on octadecylsilane-bonded silica gel (ODS) HPLC using an acetonitrile/methanol/water (TOSOHASS ODS-80TS; 10 μm, 21.5 x 30 cm. Solvent system: solvent A: acetonitrile/methanol/water 25:5:65, solvent B: acetonitrile/methanol/water 65:5:30. Gradient: start at 0 min with solvent A and hold for 25 min. Then increase solvent B to 35% over 50 min. Flow = 6.0 mL/min). All fractions yielded approximately the same HPLC profile with the activity located at two regions: peak A (t ~ 55-63 min) and peak B (t ~ 65-70 min).

Peak B was further fractionated on another reversed-phase HPLC column (Phenomenex Luna Phenyl-Hexyl; 5 μm, 250 x 10 mm. Solvent system: solvent A:

acetonitrile/methanol/water 25:5:65, solvent B: acetonitrile/methanol/water 65:5:30. Gradient: start at 0 min with solvent A and hold for 15 min. Then increase solvent B to 25% over 25 min. Flow = 2.0 mL/min). One major component was isolated; however, analytical HPLC analysis indicated a high-UV absorbing contaminant that co-eluted with active metabolite. Therefore, an alternative purification method was employed (method B).

Method B

Alternatively, the homogenized cell-free whole broth culture is passed through non-ionic polymeric resin (Supelco Diaion HP-20), washed with water, and then methanol. The methanol eluate is further separated by reversed-phase HPLC (HP Zorbax Eclipse XDB-C8; 5 μm, 150 x 4.6 mm. Solvent system: solvent A: acetonitrile/methanol/water 25:5:65, solvent B: acetonitrile/methanol/water 65:5:30. Gradient: start at 0 min with solvent A and increase solvent B to 3% in 20 min. Flow = 0.8 mL/min) to afford the same active peaks observed in method A (peaks A and B) and confirmed by analytical HPLC using UV and MS detection. An HPLC trace is shown in Figure 3.

Characteristics of active metabolites of NRRL No. B-30145

The impure fraction obtained from method A provided some initial information about the nature of the active metabolite. LC MS indicated a molecular weight $[M + H^+]$ = 892.6 and the UV spectrum displays a shoulder at 215-220 nm. 1D and 2D NMR suggests at least 2 propargyl alcohol segments [C=C-CH(OH)], several oxygenated methine carbons (X-CH-Y), and a possible sugar moiety. ^{1}H and ^{13}C NMR are shown in Figures 4 and 5 respectively.

Even though method B has not provided sufficient quantities for NMR analysis, this method yielded cleaner peaks in sufficient amounts for analysis by HPLC (octyl bonded silica gel) using UV and MS detection methods. Two major peaks (peak A and B) were obtained that matched the same compounds identified as the active metabolites using method A (see Figure 3). The UV spectra of all compounds presented a shoulder at 215-220 nm. LC MS of peak A indicated the presence of at least three (3) compounds with the following molecular weights $[M + H^+]$ = 866.5, 882.5, and 898.4 (see Figure 6). Similarly, peak B showed at least three (3)

compounds with molecular weights [M + H^+] = 892.5, 908.5, and 924.5 (see Figure 7).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

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CLAIMS

What is claimed is:

1. A metabolite produced by *Streptomyces* sp. strain NRRL No. B-30145 and mutants thereof having all the identifying characteristics of NRRL No. B-30145 and that exhibits activity against plant pathogenic fungi.

2. The metabolite of claim 1, wherein the metabolite has a molecular weight [M+ H^+] between about 925 to about 865.

3. The metabolite of claim 2, wherein the molecular weight is selected from the group consisting of 866.5, 882.5, 898.4, 892.5, 908.5 and 924.5.

4. The metabolite of claim 1, wherein the metabolite is heat and base stable, is acid labile and has a molecular weight [M+ H^+] between about 925 to about 865.

5. The metabolite of claim 4, wherein the molecular weight is selected from the group consisting of 866.5, 882.5, 898.4, 892.5, 908.5 and 924.5.

6. The metabolite of claim 1, wherein the metabolite has a chromatogram at 220 nm shown in Figure 3.

7. The metabolite of claim 1, wherein the metabolite exhibits UV absorption between about 215 nm and 220 nm.

8. The metabolite of claim 1, wherein the metabolite has a ^1H Nuclear Magnetic Resonance spectra shown in Figure 4.

9. The metabolite of claim 1, wherein the metabolite has a ^{13}C Nuclear Magnetic Resonance spectra shown in Figure 5.

10. The metabolite of claim 1, wherein the metabolite comprises one or more molecules selected from the group consisting of propargyl alcohol segments [C=C-CH(OH)], oxygenated methine carbons (X-CH-Y) or a sugar moiety.

11. The metabolite of claim 10, wherein the metabolite comprises at least two propargyl alcohol segments [C=C-CH(OH)].

12. A composition comprising the metabolite of claim 1 and a carrier.

13. A composition comprising more than one metabolite of claim 1 and a carrier.

14. The composition of claim 12, further comprising at least one chemical or biological pesticide.

15. The composition of claim 13, further comprising at least one chemical or biological pesticide.

16. The composition of claim 12, wherein the composition is formulated from the group consisting of a wettable powder, a granule, an aqueous suspension, and emulsifiable concentrate and a microencapsulated formulation.

17. The composition of claim 13, wherein the composition is formulated from the group consisting of a wettable powder, a granule, an aqueous suspension, and emulsifiable concentrate and a microencapsulated formulation.

18. A method for protecting or treating plants, fruit, and roots from fungal infections comprising applying an effective amount of the metabolite of claim 1 to the plant, fruit or root.

19. The method of claim 18, wherein the infections are caused by a fungus selected from the group consisting of *Alternaria solani, Botrytis cinerea, Rhizoctonia sp., Sclerotinia sp.*, and *Phytophthora sp.*

20. The method of claim 18, wherein more than one metabolite of Streptomyces sp. NRRL No. B-30145 strain that exhibits activity against plant pathogenic fungi is applied.

21. The method of claim 18, wherein the metabolite has a molecular weight [M+ H^+] between about 925 to about 865.

22. The method of claim 21, the molecular weight of the metabolite is selected from the group consisting of 866.5, 882.5, 898.4, 892.5, 908.5 and 924.5.

23. The method of claim 18, wherein the metabolite is heat and base stable, is acid labile and has a molecular weight [M+ H^+] between about 925 to about 865.

24. The method of claim 23, wherein the molecular weight is selected from the group consisting of 866.5, 882.5, 898.4, 892.5, 908.5 and 924.5.

25. The method of claim 18, wherein the metabolite has a chromatogram at 220 nm shown in Figure 3.

26. The method of claim 18, wherein the metabolite exhibits UV absorption between about 215 nm and 220 nm.

27. The method of claim 18, wherein the metabolite has a ^{1}H Nuclear Magnetic Resonance spectra shown in Figure 4.

28. The metabolite of claim 18, wherein the metabolite has a ^{13}C Nuclear Magnetic Resonance spectra shown in Figure 5.

29. The method of claim 18, wherein the metabolite is applied as a formulation selected from the group consisting of wettable powders, granules, aqueous suspensions, emulsifiable concentrates or microencapsulations.

30. The method of claim 29, further comprising applying an effective amount of at least one chemical or biological pesticide.

31. The method of claim 29, wherein the formulation comprises more than metabolite.

32. An antifungal composition comprising a metabolite produced by Streptomyces and isolated according to a method comprising:

(a) loading a whole broth culture of *Streptomyces sp. strain* NRRL No. B-30145 or mutants thereof having all the identifying characteristics of NRRL No. B-30145 onto a non-ionic absorbent polymeric resin;

(b) eluting the metabolite with an alcohol;

(c) screening the eluent of step (b) with a bioassay for fractions of the eluent exhibiting antifungal activity;

(d) loading the fractions of the eluent exhibiting antifungal activity of step (c) on a HPLC column; and

(e) eluting the metabolite with an organic solvent.

33. The composition of claim 32, wherein the eluent of step (b) is methanol or a gradient of aqueous methanol.

34. The composition of claim 32, wherein the bioassy of step (c) is selected from the group consisting of the agar diffusion assay or slide germination assay.

35. The composition of claim 32, wherein the organic solvent of step(e) is an acetonitrile –water gradient.

36. A method for protecting or treating plants, fruit, and roots from fungal infections comprising applying an effective amount of the composition of claim 32 to the plant, fruit or root.

37. The method of claim 32, wherein the infections are caused by a fungus selected from the group consisting of *Alternaria solani, Botrytis cinerea, Rhizoctonia sp., Sclerotinia sp.*, and *Phytophthora sp.*

38. The method of claim 32, wherein the *Streptomyces sp.* strain NRRL No. B-30145 is applied as a formulation selected from the group consisting of wettable powders, granules, aqueous suspensions, emulsifiable concentrates or microencapsulations.

39. The method of claim 32, further comprising applying an effective amount of at least one chemical or biological pesticide.

Abstract

A novel antibiotic-producing *Streptomyces* sp. is provided that exhibits antifungal activity only on certain specific plant pathogens. Also provided is a method of treating or protecting plants from fungal infections comprising applying an effective amount of an antibiotic-producing *Streptomyces* sp. having all the identifying characteristics of NRRL Accession number B-30145. The invention also relates to fungicidal compositions comprising this novel *Streptomyces* strain and the antibiotics and metabolites produced by this strain either alone, or in combination with other chemical and biological pesticides.

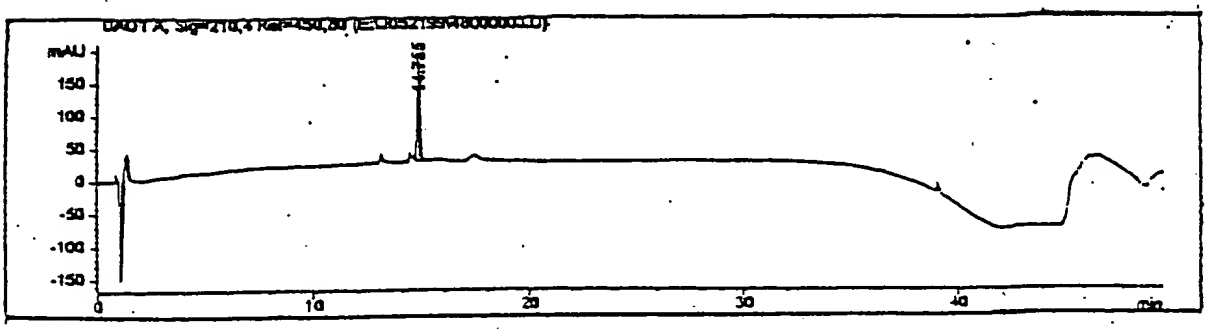

Figure 1A

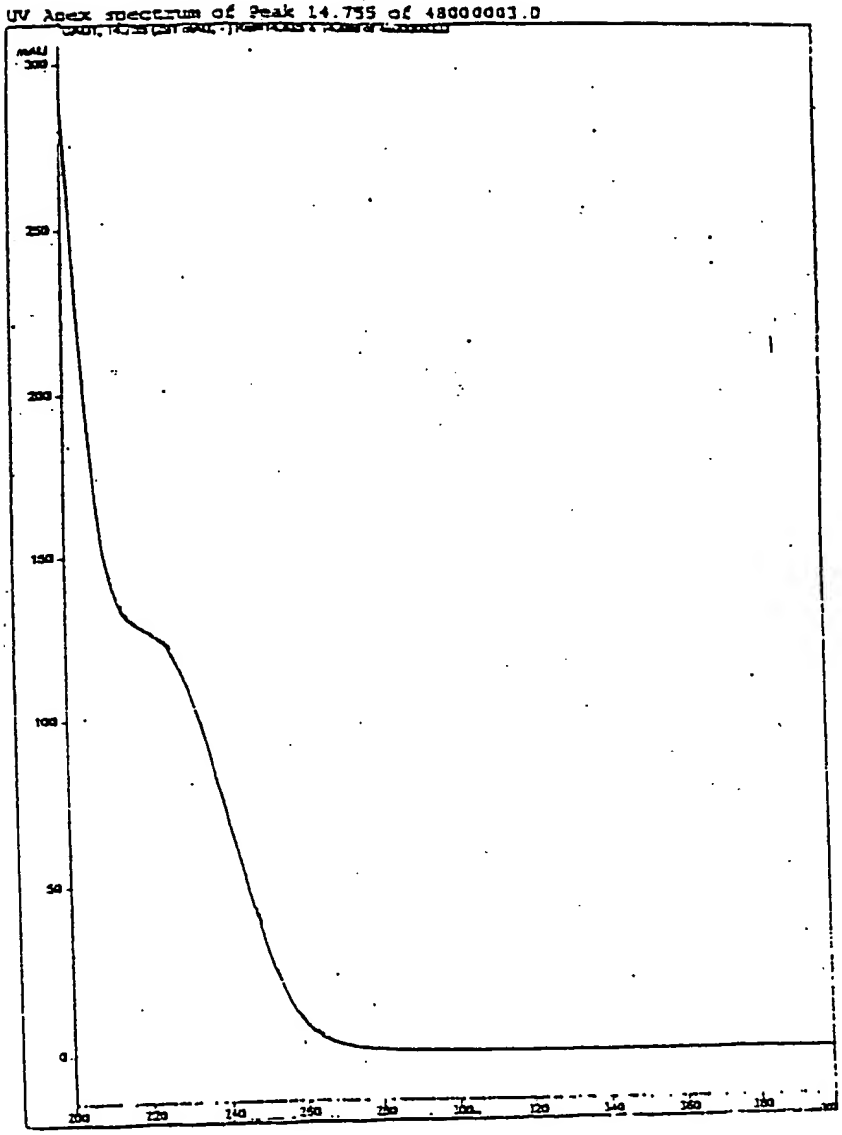
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Figure 1B

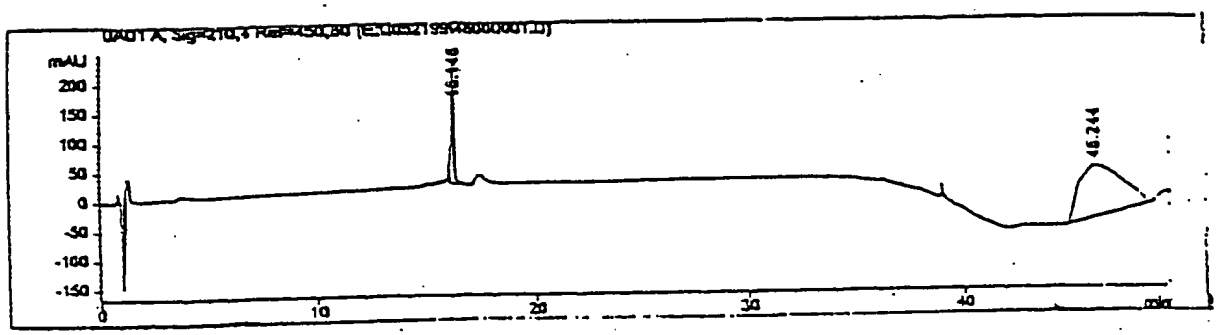

Figure 2A

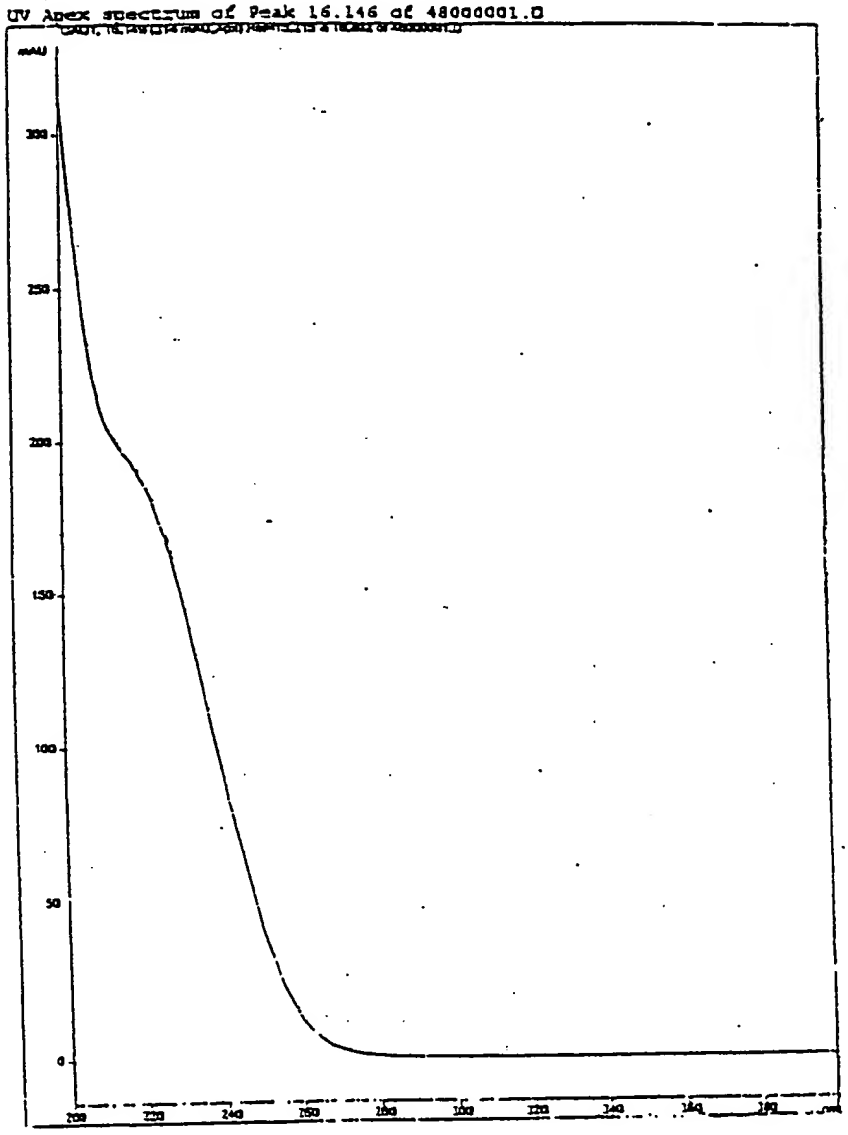
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Figure 2B

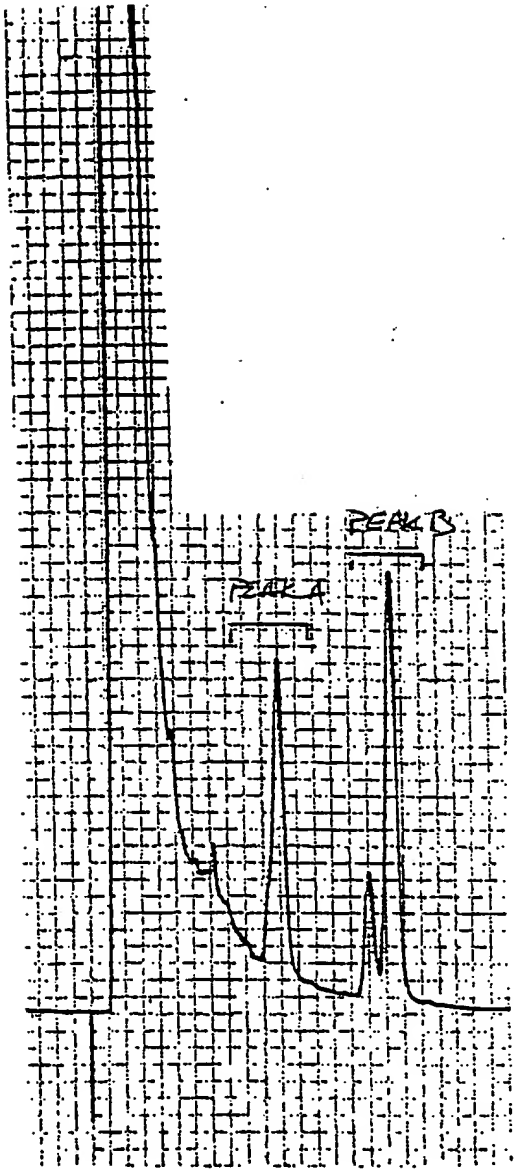


Figure 3

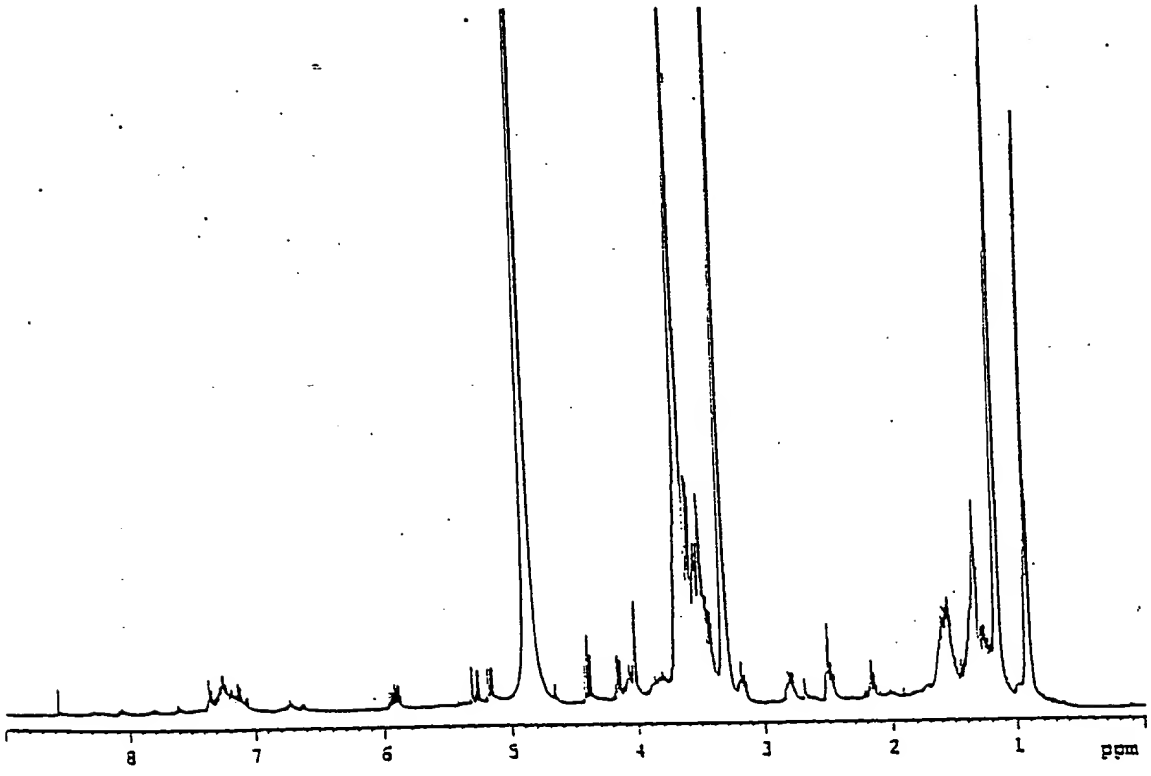
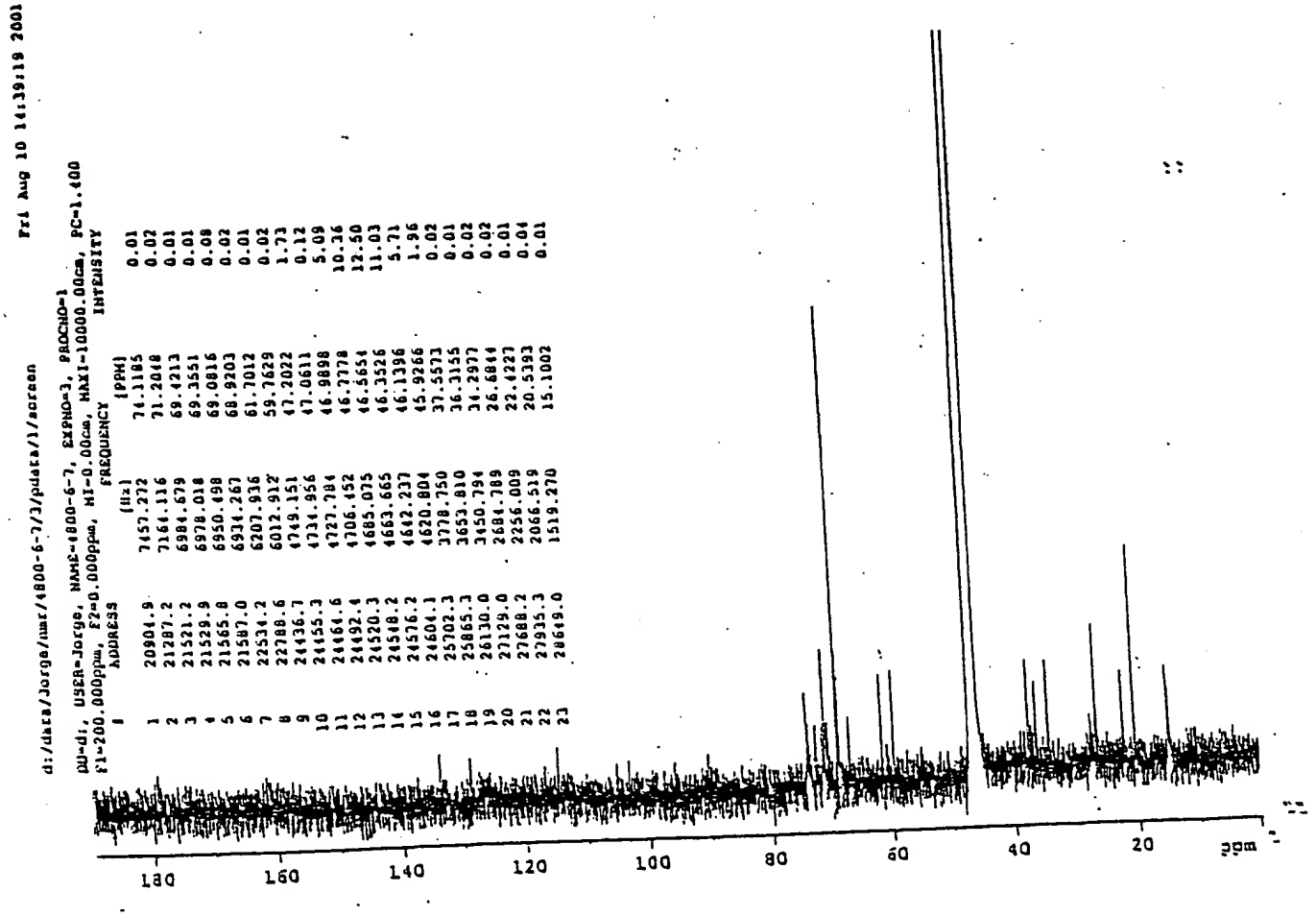


Figure 4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(PPM)
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2 21287.2 6984.679 69.4213 0.01
3 21521.2 6978.018 69.3551 0.01
4 21529.9 6950.498 69.0816 0.08
5 21565.8 6931.267 68.8203 0.02
6 21587.0 6207.936 61.7012 0.01
7 22534.2 6012.912 59.7629 0.02
8 22788.6 4749.151 47.2022 1.73
9 24436.7 4734.956 47.0611 0.12
10 24455.3 4727.784 46.9898 5.09
11 24464.6 4706.452 46.7778 10.36
12 24492.4 4685.075 46.5654 12.50
13 24520.3 4663.665 46.3526 11.03
14 24548.2 4642.237 46.1396 5.71
15 24576.2 4620.804 45.9266 1.96
16 24604.1 3778.750 37.5573 0.02
17 25702.3 3653.810 36.3155 0.01
18 25865.3 3450.794 34.2977 0.02
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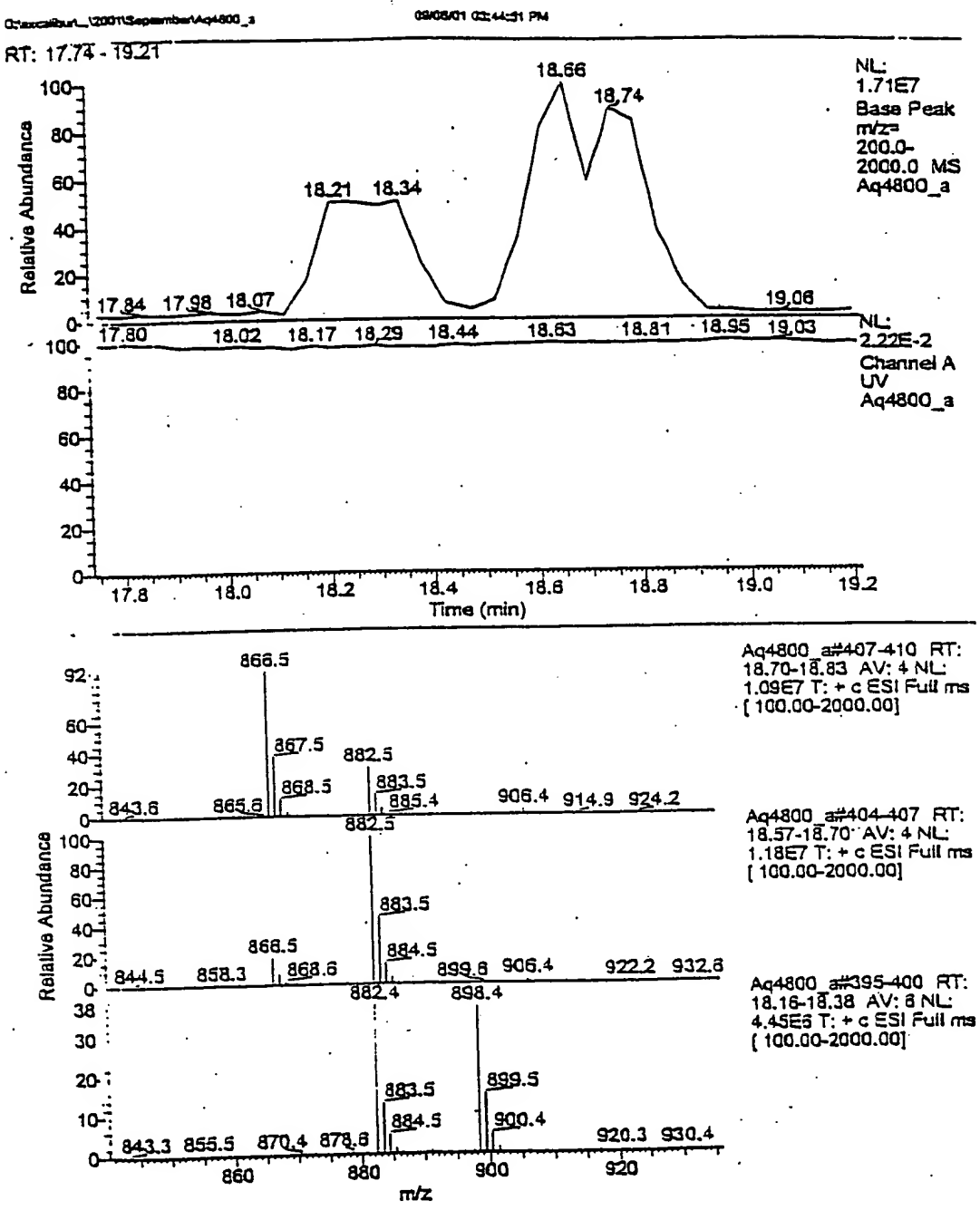
Figure 5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Relative Abundance
Time (min)
NL: 1.71E7 Base Peak m/z= 200.0-2000.0 MS Aq4800_a
NL: 2.22E-2 Channel A UV Aq4800_a
Aq4800_a#407-410 RT: 18.70-18.83 AV: 4 NL: 1.09E7 T: + c ESI Full ms [100.00-2000.00]
Aq4800_a#404-407 RT: 18.57-18.70 AV: 4 NL: 1.18E7 T: + c ESI Full ms [100.00-2000.00]
Aq4800_a#395-400 RT: 18.16-18.38 AV: 6 NL: 4.45E6 T: + c ESI Full ms [100.00-2000.00]
m/z

Figure 6

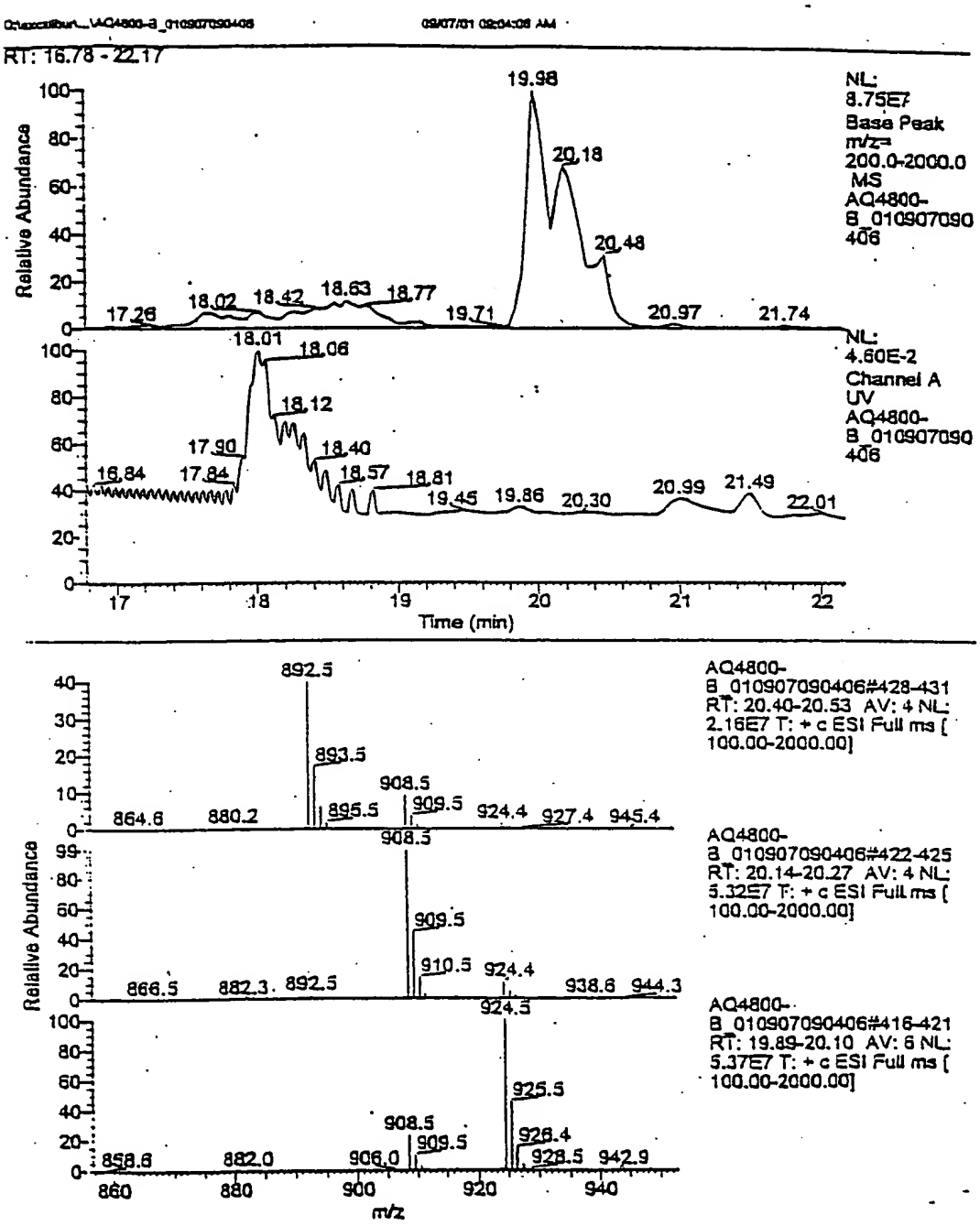
RT: 16.78 - 22.17
Relative Abundance
Time (min)
NL: 8.75E7 Base Peak m/z= 200.0-2000.0 MS AQ4800-B_010907090406
NL: 4.60E-2 Channel A UV AQ4800-B_010907090406
AQ4800-B_010907090406#428-431 RT: 20.40-20.53 AV: 4 NL: 2.16E7 T: + c ESI Full ms [100.00-2000.00]
AQ4800-B_010907090406#422-425 RT: 20.14-20.27 AV: 4 NL: 5.32E7 T: + c ESI Full ms [100.00-2000.00]
AQ4800-B_010907090406#416-421 RT: 19.89-20.10 AV: 6 NL: 5.37E7 T: + c ESI Full ms [100.00-2000.00]
m/z

Figure 7